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DEPARTMENT OF THE ARMY
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PYRUVATOKINASE, 6-PHOSPHOGLUCONATE DEHYDROGENASE,
AND ATP-ASECA ACTIVITY OF RETICULOCYTES AND NORMOCYTES

Following is the translation of an article by F. Belfiore and J. Meldolesi in the Italian-language periodical Bollettino della Societa Italiana di Biologia Sperimentale (Bulletin of the Italian Experimental Biology Society), Naples, Vol 39, No 23, 1963, pages 1574-1577.

From the Institute of Medical Pathology of Catania University.
Catania section -- 26 July 1963 session.

The study of the behavior of enzyme activities in erythrocytes during the first phases of their live development is of considerable interest both from the viewpoint of the physiology of the red corpuscles and also because it enables us to make a more exact interpretation of the enzyme changes very often observed in the erythrocyte under pathological conditions accompanying reticulocytosis.

In the course of a systematic investigation on the enzyme inheritance of reticulocytes, we were able to demonstrate an increase in the activity-- in the mature erythrocyte--of malic dehydrogenase (1), of glutamic-oxalacetic and glutamic-pyruvic transaminase and of arginase (2), of phosphohexose isomerase, glucose-6-phosphate dehydrogenase, aldolase, and lactic dehydrogenase (3), of isocitric dehydrogenase (4), of the content of ATP (5) and reduced glutathione (6), while we did not encounter any change in the acid phosphatase activity (7). In this report we are concerned with the behavior of pyruvate kinase, 6-phosphogluconate dehydrogenase, and ATP-ase activity.

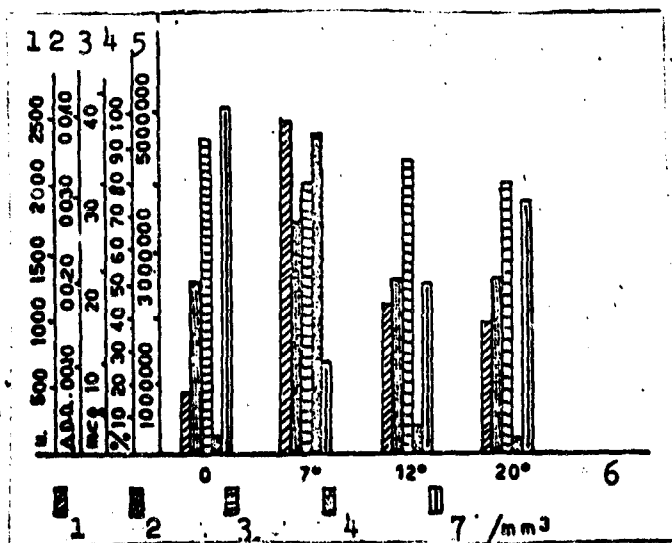
An increase in 6-phosphogluconate dehydrogenase has been described in young human erythrocytes selected by means of fractionated centrifugation or differential osmotic lysis (8) and in rabbit erythrocytes in the post-hemolytic reticulocytosis phase (9); in this latter experimental condition an increase in ATP-ase was observed (10).

Materials and methods. The investigation was conducted on two groups of rabbits of both sexes with an average weight of 1.5 kg. In the animals in the first group, we obtained intensive reticulocytosis leading to a hemolytic crisis by means of daily injections of acetylphenylhydrazine (10 mg/kg by weight) for 5 days; in the animals in the second group we obtained the same results with a daily blood-letting (about 25 ml) for 5 days. Before the start of treatment, that is, after 7, 12, and 20 days, we took blood samples by means of intracardiac puncture, using a heparanized syringe. In the blood thus obtained we made a count of red blood corpuscles and reticulocytes which were dyed with brilliant-blue of cresyl; we then determined the enzymes on the hemolysate by adding 4 volumes of distilled water to one volume of red corpuscles, washed and centrifugated at 3,000 rpm for 10 minutes.

Pyruvatokinase was determined by the method of Bock and his associates (11), expressed in units and related to 1 ml of hemolysate diluted 1/5.

The 6-phosphogluconate dehydrogenase was determined by the method of Marks (12), expressed in Δ D. 0./5 minutes and related to 1 ml of hemolysate diluted 1/20. The ATP-ase was determined by means of the method of Bucher and Schuart (13), measuring the residual ATP after incubation of the hemolysates for a period of 2 hours in a solution of ATP at the previously mentioned dilution. The results are expressed in mug of separated ATP.

Results. The results for the first group of animals are summarized in the following figure. We see that, under basic conditions, the red corpuscles numbered 5,270,000 per cu mm, the reticulocytes were 2.7%, the pyruvatokinase activity was equal to 465 u, the 6-phosphogluconate dehydrogenase activity was 0.020 Δ D.O., and the ATP-ase activity was 34 mug of ATP separated during 2 hours of incubation.



Pyruvatokinase, phosphogluconate dehydrogenase, and ATP-ase activity of erythrocytes in rabbit during experimental hemolytic anemia. Legend: (1) pyruvatokinase; (2) 6-phosphogluconate dehydrogenase; (3) ATP-ase; (4) reticulocytes; (5) erythrocytes; (6) days of treatment; (7) red blood corpuscles.

On the 7th day--that is at the moment of maximum anemization (1,420,000 red corpuscles)--we had a serious reticulocytosis, amounting to 95%, to which corresponded a pyruvatokinase activity of 2,500 U, a 6-phosphogluconate dehydrogenase activity of 0.027 D.O., and a ATP-ase activity of 30 mug of ATP. These values -- with respect to the basal values revealed in the normocytes-- indicate a statistically significant increase of 437% for pyruvatokinase ($P < 0.001$), of 35% for 6-phosphogluconate dehydrogenase ($P < 0.02$), and a slight (-8.5%), statistically not significant change in ATP-ase. In the later phases of the experiments, as the hematological conditions observed on the 12th and 20th days (2,511,000 and 3,580,000 red corpuscles; 7.2 and 2.5% reticulocytes, respectively) improved progressively, we also had a rapid decrease in the pyruvatokinase activity (1,850 and 1,000 U) as well as in 6-phosphogluconate dehydrogenase activity. This latter activity was in the neighborhood of the original values (0.021 and 0.0215 Δ D.O.). Finally, the ATP-ase remained in the vicinity of the basic values (33 and 30.6 mug of ATP).

The second group of animals, where reticulocytosis was produced by means of bleeding, was studied in order to determine whether the enzyme changes observed in the first group of rabbits were influenced by the toxic action of the hemolyzing substance. Starting with basic conditions identical to those described above, we observed 2,593,000 erythrocytes on the 7th day, with 41% reticulocytes; pyruvatokinase had increased 262% ($p < 0.001$), and reached 1,675 U; the 6-phosphogluconate dehydrogenase increased 22% ($p < 0.01$), and reached 24.4 Δ D.O.; the ATP-ase also revealed a small increase of 13%.

On the 12th day, we had 2,765,000 erythrocytes of which 5% were reticulocytes; pyruvatokinase had increase 169% (1,250 U), the 6-phosphogluconate dehydrogenase had returned to normal values and the ATP-ase revealed a very low activity of 17.6 mug of ATP. Considering the small degree of reticulocytosis encountered in the second group of animals, these data are sufficiently in agreement with those described for the first group of animals except for the slight increase in ATP-ase in animals anemized by bleeding.

We also calculated the coefficient of correlation between the reticulocyte rate and the individual activities in order to determine whether the enzyme changes encountered in the course of the experiment demonstrated a development parallel to the variation in the percentage of reticulocytes or whether they would continue even after the drop in the reticulocyte rate. This coefficient turned out highly significant for 6-phosphogluconate dehydrogenase ($p < 0.001$) while it was not significant for pyruvatokinase and ATP-ase. From this we can deduce that the increase in the 6-phosphogluconate dehydrogenase activity constitutes a characteristic of the young, morphologically reticulated erythrocyte, while the increase in the pyruvatokinase activity continues in the still young erythrocytes which, however, have lost

their granular and filamentous substance.

Finally, if we consider that there is a certain ratio of mature elements present also in the phase of maximum reticulocytosis, it is obvious that the data given above indicate the activity of a mixture of reticulocytes and normocytes. Knowing the activity of the normocytes from the study conducted under basic conditions, we were able to use a simple mathematical method in calculating the activity of the reticulocytes which, in the case of pyruvate kinase, turned out to be 2,717 U and, in the case of 6-phosphogluconate dehydrogenase, 0.0274 Δ D.O.

We can therefore conclude the following: While ATP-ase does not, in the reticulocyte, reveal an activity noticeably different from that of the mature erythrocyte, the 6-phosphogluconate dehydrogenase and the pyruvate kinase reveal a noticeably increased activity. The 6-phosphogluconate dehydrogenase activity, corresponding to the disappearance of the granular and filamentous substance, moves toward the values that are characteristic of the normocyte; this gives ~~us~~ a noteworthy correspondence between the behavior of the enzyme and the morphological aspect. Pyruvate kinase, instead, decreases more slowly and maintains a still rather high activity in the young erythrocytes which are no longer reticulated.

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